

## Characterization of Male Goat Odors: 6-Trans Nonenal

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### ABSTRACT

Solvent extraction and nitrogen degassing were employed to collect volatile odors from the lipid complex yielded by swabbing the sebaceous gland areas of the scalps of sexually active bucks from four breeds of goats. Gland tissue samples from freshly slaughtered bucks and does also were obtained for lipid analysis. Identified as the major compound responsible for one of the characteristic odors of the buck was 6-trans nonenal. Isolation of the compound as its 2,4-dinitrophenyl hydrazone was achieved by column and thin-layer chromatography. Tentative identification was based on comparison with known compounds by argentation thin-layer chromatography, retardation factors for the 2,4-dinitrophenyl hydrazone derivatives, and gas-liquid chromatography retention times of regenerated compounds. The presence of other nonenals suggests that the compound may be formed by oxidation of the gland lipids.

The odor of the male goat in rut often is implicated as a source of the "goaty" flavor problem in fresh goat milk. When added to goat milk, 6-trans nonenal yielded a significant melon-musky flavor response from the flavor panel; the "goaty" flavor response was more significant for added 4-ethyl octanoic acid, 4-methyl octanoic acid, and 4-ethyl oct-2-enoic acid.

### INTRODUCTION

The male goat (buck) is notorious for generating offensive odors during the rutting

season and often is implicated as an external factor in causing "goaty" flavored milk on the farm (1). The high volatility of buck odors has led to a recommended practice of housing the buck in an area isolated some distance from the milking area and doe housing area (1). The buck in rut sprays urine over its chin and neck area, and although this urine has a distinct odor, the dominant buck odors are attributed to secretions of the sebaceous scent glands (2, 3). These glands are located caudomedial to the aspect of the base of the horns (2), and they hypertrophy during the breeding season. They are in female goats (does) as well but are not usually active in the does.

Because these odorous secretions are believed to possess certain pheromonal activity, they often are swabbed from the glandular area of the buck by goat breeders. The "buck rag" is hung in the presence of buck-less doe herds as an aid in inducing estrus. Veterinarians and agriculture extension workers recommend this practice, but researchers conducting the only scientific study we found (14) used buck urine rather than glandular secretions; results were inconclusive. One odor, suspected by the authors as being a nonenal, often can be detected several hundred meters from the goat farm during the rutting season. Our efforts in establishing 6-trans nonenal as a major contributor to this odor are summarized in this report.

### MATERIALS AND METHODS

#### Sample Collection

Cheesecloth was detergent washed, acid washed, rinsed until neutral, dried, and extracted in a Soxhlet extractor with carbonyl-free hexane for 6 h prior to being cut into .25 m<sup>2</sup> swabs for collection of glandular secretion. Samples were collected from Alpine, Nubian, Saanen, and Toggenburg bucks from five farms in PA and NJ over two breeding seasons. After vigorous swabbing of the area behind the horns and down into the back of the neck, each swab was placed in a tightly closed sample jar, stored

in an ice bath, and extracted within 4 h of sampling. Samples of freshly slaughtered doe and buck scalps and buck urine were obtained from a local slaughterhouse and stored at  $-40^{\circ}\text{C}$  until extracted.

#### Solvent Purification

Hexane, benzene, and petroleum ether (b.p.  $50^{\circ}\text{C}$ ) were rendered carbonyl free by the method of Schwartz and Parks (8) and stored over calcium hydride. Methylene chloride was redistilled in glass and stored over calcium hydride. Ethyl ether was distilled freshly over sodium hydroxide as needed.

#### Solvent Extraction of Swab Samples

Solvent extraction of swabs was accomplished by addition of 100 ml of carbonyl-free hexane (all solvents referred to hereafter were purified as above) to the sample jar, shaking 1 min, and decanting the solvent. A second 100-ml extraction was combined with the first and the total extract dried over sodium sulfate. Each sample extract then was passed through individual columns containing 4 g of Analytical Grade Celite onto which 2.4 ml of saturated aqueous sodium metabisulfite had been ground (6). The packing was rinsed with 50 ml of hexane, extruded from the column, and the bisulfite decomposed with 100 ml of 10% sodium carbonate in a separatory funnel. Aldehydes liberated from the bisulfite adducts were extracted with two 50-ml portions of hexane and derivatized with 2,4-dinitrophenyl hydrazine (DNP-hereafter refer to dinitrophenyl) on a reaction column (8).

#### 2,4-DNP Hydrazone Chromatography

The 2,4-DNP hydrazones in hexane were reduced to dryness under nitrogen on a steam bath, taken up in a minimum of benzene, and separated by thin-layer partition chromatography (TLC) with an alkaline stationary phase according to the method of Schwartz et al. (10). After developing in hexane, 2,4-DNP hydrazones on the plate were divided into seven bands (Figure 1) that were scraped from the plates, and a portion of each was regenerated with a few drops of 10% aqueous phosphoric acid in a sealed vial at  $95^{\circ}\text{C}$  for odor evaluation. Bands 2 and 3 were odor significant, and the 2,4-DNP hydrazones were freed from the

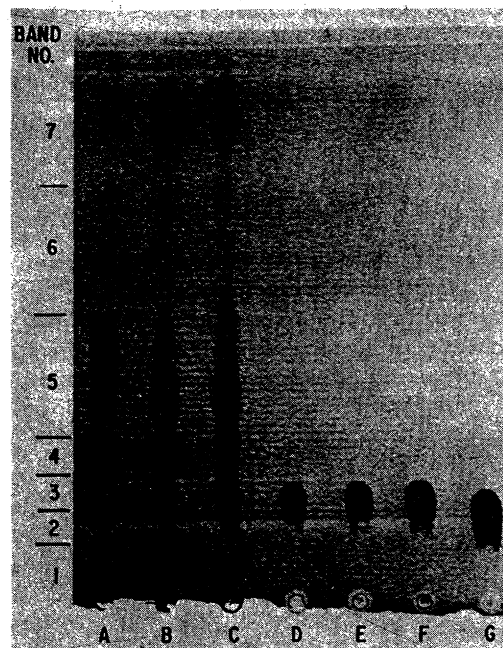


Figure 1. Separation of aldehyde 2,4-dinitrophenylhydrazones by thin-layer chromatography on an alkaline polyethylene glycol 400 stationary phase and Microcel T-38 support. Solvent system-hexane. A, Alpine buck sample; B, Nubian buck sample; C, Saanen buck sample; D, 4-trans nonenal; E, 5-trans nonenal; F, 6-trans nonenal; G, 6-cis nonenal.

Microcel support by eluting with benzene through glass wool filters. After benzene was removed under nitrogen, fractions were redissolved in hexane and passed over small columns of 6%  $\text{H}_2\text{O}$ -deactivated neutral alumina to remove the polyethylene glycol 400.

Portions of the purified 2,4-DNP hydrazone bands were chromatographed along with 2,4-DNP hydrazone reference standards of 4-trans, 5-trans, 6-trans, 7-trans, and 6-cis nonenals on silica gel plates impregnated with silver nitrate according to Parks et al. (6). Individual sample spots along with standards were compared for  $R_f$ , scraped from the plates, removed from silica gel with benzene, and regenerated for gas-liquid chromatography (GLC) and odor evaluation.

#### Solvent Extraction of Glandular Tissue

Gland areas of scalps excised from freshly slaughtered bucks and does were trimmed free of hair, extraneous matter, and adhering

muscle tissue, and cut into 1-mm<sup>3</sup> portions. Two to five grams of sample were ground in a 15.24 cm mortar with 1 ml of 10% phosphoric acid, followed by 10 g of sodium sulfate and 15 g of Celite 545.<sup>2</sup> This material was tamped into a 3-cm i.d. glass column plugged with purified glass wool (13), and the lipid material was eluted with 200 ml of methylene chloride and dried over sodium sulfate. The extract was reduced to a 50-ml volume under nitrogen at room temperature and subjected to bisulfite adduction or analyzed for other lipid materials.

#### Nitrogen Extraction of Volatiles

As a check against possible formation of artifacts during 2,4-DNP hydrazone formation, duplicate swabs from two bucks and discarded hair and scalp tissue from a slaughtered buck were degassed with high-purity nitrogen in the apparatus of Figure 2. Nitrogen under slight pressure was diffused through fritted glass at the bottom of the flask and dispersed through the finely cut sample material to elute volatiles. The extracted volatiles were adsorbed onto a 2-g Tenax GC (Applied Science, State College, PA) trap mounted on the suction side of a permissible air supply pump (Bendix Environmental Science Division, Baltimore, MD) at a flow rate of 500 cm<sup>3</sup>/min for 8 h. Volatiles were eluted from the trap with 5 ml of petroleum ether followed by 5 ml of ethyl ether; the combined extract was dried with sodium sulfate, then evaporated to about 100  $\mu$ l under nitrogen for GLC.

#### Regeneration of 2,4-Dinitrophenyl Hydrazones for Gas-Liquid Chromatography

Approximately 5  $\mu$ g of each derivative dissolved in 10  $\mu$ l of benzene was transferred to a melting point capillary tube sealed at one end (16), and the solvent was removed with vacuum. Ten microliters of freshly prepared 2% HCl in acetone was added, stirred with a fine stainless-steel wire, and the mixture was capped with foil and allowed to stand for 30 min. A small

compact bed (2-cm long) of MgO:analytical grade Celite (1:1) was constructed in a melting point capillary. Purified glass wool was tamped onto the top and bottom of the packing material, and the sample was adsorbed onto the packing. Two 10- $\mu$ l injections of hexane were used to elute the regenerated, purified aldehyde, and the first 5 to 10  $\mu$ l was collected for injection into the gas chromatograph. The odor of the aldehyde could be evaluated readily by our smelling the syringe plunger after injection.

#### Gas-Liquid Chromatography

Peak retention times were established for regenerated 2,4-DNP hydrazone standards on two gas chromatographic systems equipped with flame ionization detectors. A Shimadzu GC-MINI 2 gas chromatograph was equipped with a 12-m OV101 fused silica capillary column (Hewlett Packard Co., Avondale, PA) and a helium gas flow of 51 ml/min, split 50:1 after injection and ahead of the column. Samples were run isothermally at 65°C, and retention times relative to nonanal were cal-

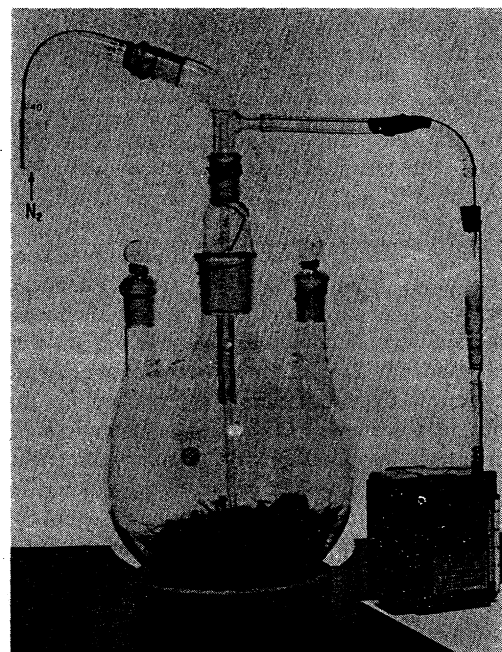


Figure 2. Degassing apparatus for trapping volatile buck odors on a Tenax GC trap, mounted on a permissible air supply pump.

<sup>2</sup> Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

culated. A Hewlett Packard model 5750 gas chromatograph with a 1.83 m x .64 cm o.d. stainless-steel column packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on Anakrom AS/90/100 mesh (Analabs, North Haven, CN) and 20 ml/min helium carrier gas was programmed from 65°C to 220°C at 4°/min to evaluate odors from the Tenax GC trap and regenerated 2,4-DNP hydrazone spots. After we established retention times for the known standards of the nonenals, the flame was extinguished after the solvent peak was eluted from each sample injection, and the retention time of significant odors was recorded as they were smelled at the detector.

#### **Generation of 6-Trans Nonenal from Gland Lipid**

Approximately 30 mg of the methylene chloride extracted fat from a buck gland free from detectable (chemically or organoleptically) 6-trans nonenal was transesterified by modification of the procedure of Luddy et al. (5) for fats high in free fatty acids. Reaction time of the fat with sodium methoxide in the water bath was increased from 2 to 20 min because of the high concentration of sterol esters in the sample, and petroleum ether was substituted for CS<sub>2</sub> as the extracting solvent for recovering the methyl esters. Transesterification was determined complete by routine TLC on silica gel plates developed in 80:20:1 hexane:ethyl ether:acetic acid, and spots were revealed by charring with 50% aqueous sulfuric acid and heat. The methyl ester mixture was concentrated under N<sub>2</sub> at room temperature in a glass vial and reduced with 3 drops of vitride [sodium dihydride-bis (2 methoxyethoxy) aluminate 70% in toluene) (Alfa Products, Danvers, MA)] followed by 3 drops of benzene. The vial was sealed, and the sample was allowed to stand for 2 h at room temperature before reduction to alcoholates was checked on the routine silica gel TLC system. Alcohols were recovered by passing the reaction mixture in benzene over a column prepared by grinding 10 g of Analytical Grade Celite with 6 ml of phosphoric acid and 3 ml of distilled water prior to tamping into the glass column. Regenerated alcohols eluted from the column in benzene were dried under nitrogen, redissolved in methylene chloride, and oxidized to the aldehydes by passing them over a chromic

acid-celite column method as described by Schwartz and Osman (12). The mixture then was passed through the 2,4-DNP hydrazone reaction column followed by a MgO:Celite column to remove lipids and a weak alumina column to isolate the monocarbonyls by the procedure of Schwartz et al. (9). Separation and identification of 2,4-DNP hydrazones was as before.

#### **Flavor Evaluation**

Fresh pasteurized goat milk, free from "goaty" flavor, was divided into five lots for evaluating the effect of added compounds in producing "goaty" flavored milk. Compounds added and amounts are in Table 1. Eight experienced panelists evaluated samples for goaty, rancid, melon-musky, or other flavor criticisms on a scale of 0 to 4, 0 none, 1 questionable, 2 slight, 3 distinct, and 4 strong. Panel evaluations were analyzed by standard analysis of variance procedures and Duncan's multiple range test.

### **RESULTS AND DISCUSSION**

Because of interfering barnyard odors, direct air sampling on the farms to trap the nonenal odor was abandoned in favor of swabs of the glandular secretion of the bucks. Nonenals of significance for odor were identified by extraction of these swabs and other methods of isolation (Table 2). The 5-, 6-, and 7-trans, and 6-cis nonenals are similar to the odor detected on farms, but 6-cis nonenal was not recovered in any samples; the evidence indicates that 6-trans nonenal is the most likely contributor of this odor.

Retention times by GLC of isolated nonenals were in agreement with reference compounds (Table 3), but the clean separation achieved by argentation TLC of the 2,4-DNP hydrazones (Figure 3) was necessary to confirm identity of closely eluting GLC peaks. Detection of the nonenals from nitrogen extraction of volatiles confirmed that the nonenals were not artifacts of the 2,4-DNP hydrazone chemistry. Although specificity of bisulfite for aldehydes has been reported (11) and seemed reliable for this study, the recovery of the regenerated nonenals by extraction of the bisulfite adducts was judged to be only 65% complete by these methods.

TABLE 1. Panel<sup>1</sup> means for flavor responses in goat milk adulterated with compounds associated with goat odor.

Sample adulterant	Added compound	Flavor response and means			
		Goaty	Rancid	Melon musky	Other <sup>2</sup>
	(ppm)				
4-ethyl octanoic acid	7.0	3.13 <sup>a</sup>	1.25 <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>
4-methyl octanoic acid	7.0	2.44 <sup>ab</sup>	1.00 <sup>a</sup>	.38 <sup>b</sup>	.25 <sup>ab</sup>
4-ethyl oct-2-enoic acid	2.5	1.75 <sup>bc</sup>	1.00 <sup>a</sup>	0 <sup>b</sup>	.50 <sup>ab</sup>
6-trans nonenal	2.0	.63 <sup>cd</sup>	0 <sup>a</sup>	3.38 <sup>a</sup>	0 <sup>b</sup>
Control	0	.25 <sup>d</sup>	0 <sup>a</sup>	0 <sup>b</sup>	.88 <sup>a</sup>

<sup>a,b,c,d</sup> Means for the same flavor response with the same letter are not significantly ( $P < .05$ ) different by Duncan's multiple range test.

<sup>1</sup> Eight experienced panelists instructed to score the intensity of each flavor response by the following scale: 0 = none; 1 = questionable; 2 = slight; 3 = distinct; 4 = strong.

<sup>2</sup> Other criticisms included cooked, oily, cardboard.

The 2,4-DNP hydrazones of the nonenal reference compounds colored tan on the polyethylene glycol 400 plate (Figure 1) and ran between heptanal and octanal, which agrees

with liquid column methods reported in isolating these compounds (4, 6). The alkaline polyethylene glycol 400 plate not only gives separation of the C-5 through C-18 aldehydes but also

TABLE 2. Sampling protocol and significant nonenals identified in samples.

Breed sample #	Method of sampling				Trans nonenals identified 4 5 6 7
	Solvent extracted swab	N <sub>2</sub> extracted swab	N <sub>2</sub> extracted hair	Solvent extracted tissue	
Alpine 1	+				---+
Alpine 1 R <sup>a</sup>	+				---+
Alpine 2	+				---+
Alpine 3	+				---+
Alpine 3 R <sup>a</sup>	+	+			---++
Alpine 4			+		---+
Nubian 1	+				---+
Nubian 2	+				+++--
Nubian 3 <sup>b</sup>				+	---+
Toggenburg 1	+				---+
Toggenburg 2	+				---+
Saanen 1	+				---++
Saanen 2	+	+			+++--

<sup>a</sup> Repeat sampling of the same animal 1 mo later.

<sup>b</sup> The 6-trans nonenal generated by conversion of fatty acid methyl esters to aldehydes.

TABLE 3. Relative retention times of aldehyde reference standards and nonenal odors isolated from male goat glands.

Compound or sample	Rt/Rt <sup>a</sup>	
	Column 1 <sup>b</sup>	Column 2 <sup>c</sup>
Octanal	.44	...
Nonanal	1.00	1.00
Decanal	2.10	...
5-trans nonenal	.88	1.18
6-trans nonenal	.92	1.23
6-cis nonenal	.99	1.37
7-transnonenal	1.02	1.54
5-trans spots <sup>d</sup>	.88	...
6-trans spots <sup>d</sup>	.92	...
7-trans spots <sup>de</sup>	...	1.52
Tenax trap sample <sup>e</sup>	...	1.20
		1.25
		1.50

<sup>a</sup>Rt/Rt — relative retention time based on nonanal = 1.00.

<sup>b</sup>Capillary column, 12 m fused silica OV 101, 50-1 split, 1 ml/min column flow, 65°C isothermal; Shimadzu GC-MINI 2.

<sup>c</sup>Packed column, 1.83 m by .64 cm o.d., 7.5% EGA with 2% H<sub>3</sub>PO<sub>4</sub>, 65°C to 220°C at 40/min; Hewlett Packard GC model 5750.

<sup>d</sup>Regenerated spots of 2,4-dinitrophenyl hydrazones of gland lipid with R<sub>f</sub> of corresponding standards scraped from AgNO<sub>3</sub> impregnated silica gel plates.

<sup>e</sup>Retention time established by odor detection with detector flame extinguished after solvent peak.

provides an indication of the purity of a class of 2,4-DNP hydrazones spotted on the plate, as 2,4-dienals color rose red, 2-enals color pinkish red, saturated aldehydes color tan, and methyl ketones color yellowish grey (10). Benzaldehyde, 2-nonenal, and the C-1 to C-6 saturated aldehydes did not move well, if at all on this plate, whereas on the silver nitrate plate some of these compounds could run in the area of the 4-, 5-, 6-, and 7-trans nonenal hydrazone derivatives under the right conditions of time and temperature.

Odor evaluation of regenerated bands and spots of 2,4-DNP hydrazones scraped from both TLC systems was of further assistance in identifying aldehydes of significance to the buck odor. Odor and flavor of isomeric nonenals were evaluated at Unilever in 1968 by Keppler et al. (4); their results indicated that 6-trans nonenal was 100 to 200 times more potent than the 5-trans or 7-trans nonenals, which have a similar odor. The odor of 4-trans nonenal differed from those of the 5-, 6-, and 7-trans nonenals and the buck odor investigated here. From work by Parks et al. (6), 6-trans nonenal

has a flavor threshold in milk of .07 ppb.

Despite the high volatility and potency of 6-trans nonenal, it was not detected always around breeding bucks and never detected on visits to farms outside of the breeding season or in the slaughterhouse where animals were of questionable sexual potency. Nonenal odor may be generated intermittently by the glands as a pheromone, or it could result from oxidation or photooxidation of certain precursors in the glandular secretion of sexually active bucks. The riboflavin in buck urine could provide a catalyst for these reactions as the rutting buck sprays urine around the head and neck. This urine, per se, has a phenolic-like odor and contributes to the scent of the animal. We were able to generate 6-trans nonenal in a nonenal-free slaughterhouse-lipid sample of Nubian buck by reduction of the methyl esters to alcohols and oxidation of the alcohols to aldehydes. This confirmed that the precursor for the 6-trans nonenal was in the gland lipid.

Traditionally, the C-6, C-8, and C-10 straight-chain fatty acids have been held responsible for producing the buck odor (2), but Sugiyama et

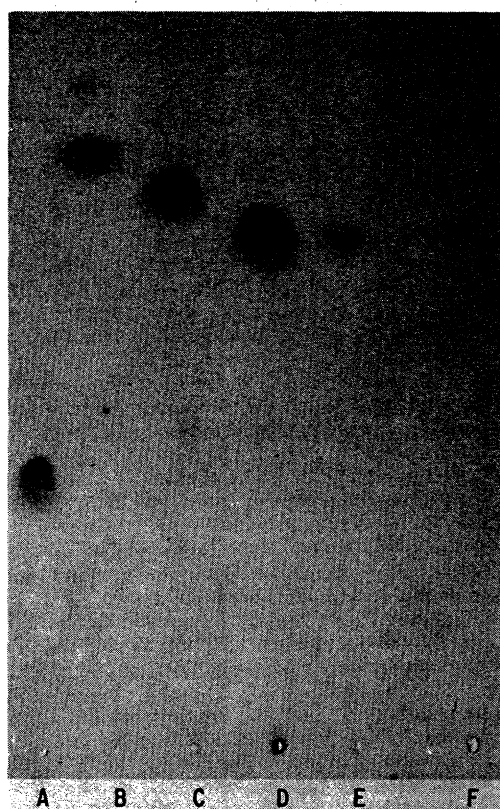


Figure 3. Separation of nonenal 2,4-dinitrophenylhydrazones on a silica gel thin-layer plate impregnated with silver nitrate. Solvent system-10% hexane in benzene. A, 6-cis nonenal standard; B, 4-trans nonenal standard; C, 5-trans nonenal standard; D, 6-trans nonenal standard; E, Alpine buck-band 2 from Microcel T-38 plate; F, aldehyde generated from buck gland free fatty acid methyl esters by reduction, oxidation procedures.

al. (15) recently isolated a homologous series of 4-ethyl branched-chain fatty acids as a major fraction of the glandular odor from native Japanese male goats. Prior to this, Wong et al. (17) reported on "goaty" flavor in wild goat meat and attributed it to 4-methyl octanoic acid. The Givaudan Company isolated 4-ethyl oct-2-enoic acid from goat milk in Switzerland (private communication) as the major flavor compound responsible for "goaty" flavored milk. From the beginning of our study it was evident that branched-chain fatty acids could account for as much as 55% of the total fatty acid profile in the scent gland lipids, but be-

cause of the complexity of the mixture, they will be reported in a separate paper. However, because the presence of a buck often is implicated as a source of "goaty" flavor in milk, it seemed appropriate to attempt a preliminary comparison of flavor responses for some of these acids and 6-trans nonenal added to a high quality goat milk.

The 6-trans nonenal (Table 1) yielded a significant melon-musky flavor response, whereas the "goaty" flavor response was significant for the branched-chain acids. Six-trans nonenal was added at a concentration well above its threshold (07 ppb) to compare with added acids for which thresholds are unknown. These additions had to be chosen arbitrarily because insufficient amounts of standards were available to determine thresholds. All added compounds were well below the thresholds of acids associated with rancidity, such as butyric (25 ppm) and caproic (14 ppm) as reported by Patton et al. (7).

Trace amounts of 6-trans nonenal and these branched-chain acids individually or synergistically could account for the musky, astringent rancidity that renders "goaty" flavor in goat milk uniquely different from the rancid off-flavor of cow milk. A thorough investigation of "goaty" flavor is beyond the scope of this paper, but the male scent glands have provided us with a natural concentration of potent biological flavor compounds that otherwise would have been difficult to isolate as trace constituents in goat milk. Routine panel evaluation of extremely "goaty" milk from farms without a buck suggests that compounds responsible for the flavor problem in goat milk must originate from sources other than the buck, although the presence of a buck could enhance the problem. Malodorous glands in does are not uncommon (3) and could be one source of the compounds in milk.

Although some research on the influence of exteroceptive factors such as odor of the male on initiation of estrus in Angora goats has been conducted by Shelton (14), the role of these odors as sex, territorial, or defense pheromones has yet to be established. If proven valuable for inducing estrus, these odors could be beneficial to goat dairy producers in helping to control the seasonal reproductive cycle of goats that is reflected in serious goat milk shortages in winter months and surpluses in summer months.

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